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SEGMENTATION OF THE OVUM, WITH ESPECIAL REFERENCE TO THE MAMMALIA.

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THERE follows after impregnation a short pause, and then the ovum begins its process of repeated division, which is known as the "*segmentation of the ovum*," the term having been introduced before it was known that each "segment" is a cell. The division or cleavage (Furchung) of ova was described by Prevost and Dumas, 1824, and again by Rusconi in 1836. By usage, the term segmentation is restricted to the production of cells up to the period of development, when the two primitive germ-layers are clearly differentiated and the first trace of organs is beginning to appear.

Segmentation nucleus. The impregnated ovum has a single nucleus which is known as the segmentation nucleus, and which is formed by the union of the male and female pronuclei.[1] It is the parent of all the nuclei subsequently found in the organism, and participates actively in the process of segmentation. It is very much smaller than the nucleus of the egg-

[1] Ed van Beneden in his first paper, Ascaris, **11**, affirmed that there was no real union of the pronuclei in the impregnated ova of that species, but Carnoy, **18**, showed that van Beneden's observations were incomplete, and Zacharias has stated, **50**, that they are so defective as to be fundamentally erroneous in regard to important phases, and he points out that in reality the eggs of Ascaris offer another proof of the actual union of the pronuclei. The impregnation in this Nematod has since formed the subject of numerous articles, see van Beneden and Neyt, **12**, Carnoy, **182**, Boveri, **15**, etc., etc.

cell before maturation; it is usually membranate, and has numerous fine granules of chromatine, *microsomata*, derived from the pronuclei; in some cases the microsomata from the male pronucleus are distinguishable from those of female pronucleus. In the rabbit the nucleus when first formed has indistinct contours, an irregular shape and a homogeneous appearance (Ed van Beneden, 8, 699,) it soon enlarges, becomes regular, and acquires a distinct, centrally situated nucleolus, (Bischoff, **14**, 50, Coste **17**, Lapin Pl. ii. fig. 4,) presumably by the gathering together of the microsomata.

The position of the nucleus is always eccentric[1] so far as known, and approximately if not exactly the same as that of the egg-cell nucleus before maturation; accordingly, the degree of eccentricity varies as the amount of yolk or deutoplasm being least in alecithal and greatest in telolecithal ova. In brief, it may be said the nucleus tends to take the most central position possible with regard to the protoplasm of the ovum. The vitelline granules are not to be regarded as protoplasm, hence their accumulation may produce a one-sided distension without, however, in the least disturbing the uniform *radial* distribution of the protoplasm. The nucleus is surrounded by protoplasm with few or no yolk grains; in telolecithal ova the perinuclear accumulation is the court of protoplasm at the animal pole.

Period of repose. After the segmentation-nucleus is formed, there occurs a pause, which lasts according to observations on several invertebrates, from half to three quarters of an hour. It is probable that a similar pause ensues in the mammalian ovum, but there are as yet no observations to show whether it occurs or not. During this period the yolk expands slightly, unless, indeed, the expansion observed is due to the influence of hardening agents[2] and the monocentric radiation, which is present when the nuclei copulate, gradually fades out, and is

[1] It is often stated that the nucleus lies exactly in the centre, but I have been unable to find a single observation to justify the statement.

[2] Van Beneden states that osmic acid produces an artificial expansion of the ovum within the zona

replaced by a dicentric radiation which marks the end of the period of repose and the commencement of the first division of the ovum.

Karyokinesis of the ovum. Segmentation is a process of indirect cell division, and nowhere are more perfect karyokinetic figures to be found than in the segmenting ovum. It is, therefore, advisable to give a general account of the changes involved in every division, but inasmuch as karyokinesis is a phenomenon by no means restricted to embryonic cells, it is not one of the special subjects of the embryologist. I shall, therefore, attempt only a summary account, following in the main, O. Hertwig, **26,** 37-38, (compare Rabl's exhaustive memoir, **37.**)

It is probable that the resting nucleus has one pole at which the connection between the reticulum of the nucleus and the surrounding protoplasm is more intimate than elsewhere, as suggested by Rabl, **38.** This pole is marked by a clearer spot outside the nucleus, close against it and much smaller than it. This clear spot becomes the centre of the radiating arrangement of the protoplasm. It was, I believe, first observed by Flemming in the eggs of Echinoderms, has been seen in *Ascaris megalocephala* by van Beneden and Neyt, **12,** and by Boveri, **15,** in Siredon by Kolliker, 28, and in other cases. It is now designated as the sphere of attraction,[1] and is seen, at least in certain phases, to contain a separate central body (centrosoma of Boveri). It is not improbable that the "sphere of attraction" is identical with the *Nebenkern* of recent German writers. In a number of instances a small part of the nucleus is seen to separate off and to lie as a distinct body, *Nebenkern,* alongside the nucleus; this body has a colorable portion which is comparable to the "centrosoma." For an account of the scattered observations on the *Nebenkern*, together with the relation of these bodies to Gaule's so-called cytozoa, see G. Platner, **34,**

[1] The history and significance of the spheres of attraction as here presented cannot, by any means, be regarded as final. The observations are few, and in *most* cases the exact history of the spheres of attraction has received no attention from investigators whatsoever.

for additional observations see Prenant, **35**, and Platner, **34a.**

The sphere of attraction divides, as does also its central body, and its two parts move to opposite sides of the nucleus. There thus appear two opposite accumulations of clear protoplasm, from each of which as a centre, astral rays or radiating lines are formed in the cell-body. Meanwhile, within the nucleus, changes go on; the threads of the intranuclear network radiate out from the the pole where the sphere of attraction lies before its division, and the chromatic substance forms a number of distinct grains. When the sphere of attraction divides and its halves go asunder, the nuclear substance preserves its radiating relation to each sphere, and as the membrane of the nucleus disappears during these changes, the final result of the transformation of the nucleus is a spindle-shaped body, the points of which rest just within the clear centre of each astral system, so that the spindle stretches from one protoplasmic mass to the other. The spindle consists of fine threads extending from pole to pole and having almost no affinity for the dyes of the histologist, a peculiarity which causes them to be known as the achromatic threads. These threads are probably always compounded of a considerable number of exceedingly fine fibrillæ, see Rabl, **38**, 21-22. The colorable substance forms a number of separate grains, each of which is united with one of the achromatic threads, and all of which lie at the same level in the centre of the spindle; when the spindle is seen from the side, the chromatine grains appear to constitute a central band or disc (Strassburger's *Kernplatte*) but when the spindle is seen endwise, the separate grains are at once recognized. The shape of the grains is variable; some authors, without sufficient observational proof, have advanced the opinion that the grains are *always* V-shaped. The spindle together with the polar accumulations of protoplasm and the two accompanying radiations constitute a so-called *amphiastere.*

The domain of the radiation extends, the two protoplasmatic centres move further apart, the nuclear spindle elongates correspondingly, and the chromatic grains of the *Kernplatte*

divide. Flemming maintains that the division is always lengthwise of the V-shaped grain, but this has been controverted by Carnoy. How the division occurs in the mammalian ovum is unknown. By division, however it is effected, the number of chromatine grains is doubled; they form two sets; one set moves toward one pole, the other towards the other pole; the grains of each set keep at the same level as they move, until they reach the end of the spindle, where they appear as a polar disc (Carnoy's couronne polaire). Next the achromatic threads of the spindle break through and are apparently drawn in towards each polar crown. There are now two nuclear masses, each near but not at the centre of a radiation, and each consisting of chromatine and achromatic substance, each mass develops into a complete membranate nucleus, but the steps of this process have yet to be followed in detail in the vertebrate ovum.

The signs of division of the protoplasm usually become visible about the time the polar crowns are formed, but when the ovum contains much deutoplasm the division may be retarded. In the plane which passes through the equator of the nuclear spindle, there appears a furrow on the surface of the ovum, which gradually spreads and deepens until it is a complete fissure around the cell, it cuts in deeper until at last only a thin stalk connects the two halves of the cell, and thereupon the stalk breaks and the cell is divided. There next ensues a pause, during which the astral rays of the protoplasm disappears in the daughter cells, and the daughter nuclei assume each the form of an ordinary resting membranate nucleus.

The external appearances of segmentation in the living ovum vary, of course, especially according to the amount and distribution of the yolk material. The appearances in holoblastic ova with very little yolk are well exemplified by *Limax campestris.* Mark's description, **32**, is, nearly in his own words, as follows:

"In Limax, after impregnation, the region of the segmentation nucleus remains more clear, but all that can be distinguished is a more or less circular ill-defined area, which is less

opaque than the surrounding portions of the vitellus. After a few moments, this area grows less distinct. It finally appears elongated. Very soon this lengthening results in two light spots which are inconspicuous at first, but which increase in size and distinctness, and presently become oval. If the outline of the egg be carefully watched, it is now seen to lengthen gradually in a direction corresponding to the line which joins the spots. As the latter enlarge, the lengthening of the ovum increases, though not very conspicuously. Soon a slight flattening of the surface appears just under the polar globules, the flattening changes to a depression (Fig. 1) which grows deeper and becomes angular. A little later the furrow is seen to have extended around on the sides of the yolk as a shallow depression, reaching something more than half way toward the vegetable or inferior pole, and in four or five minutes after its appearance the depression extends completely around the yolk. This annular constriction now deepens on all sides, but most rapidly at the animal pole; as it deepens it becomes narrower, almost a fissure. By the further deepening of the constriction on all sides there are formed two equal masses, connected by only a slender thread of protoplasm, situated nearer the vegetative than the animal pole, and which soon becomes more attenuated and finally parts. The first cleavage is now accomplished. Both segments undergo changes of form, they approach and flatten out against each other, and after a certain time themselves divide.

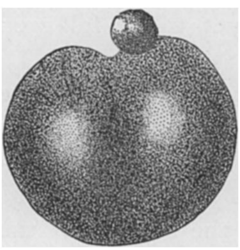


Fig. 1. Ovum of Limax campestris, during the first cleavage. Magnified 200 diameters. The envelopes are not drawn in. (After E. L. Mark.)

Primitive type of segmentation. In the lower animals there is not found that excessive amount of deutoplasm in the ovum which is so characteristic of the vertebrates, and in their ova we have what is undoubtedly the earlier and more primitive type of segmentation. In these cases the cleavage extends as in the egg of Limax, (see above) through the whole of the dividing cell. The two cells first produced are almost if not

quite alike, and each of them produces two cells which are also very similar to one another; then comes a division of the four cells into eight, four of which resemble one another and differ from the remaining cells which are also similar among themselves. Four of the cells are derived chiefly from the substance of the animal pole of the ovum and are very protoplasmic; and the other four cells are constituted out of the substance of the vegetable pole and accordingly contain most of the deutoplasm of the ovum. The eight cells form an irregular spheroid, in the centre of which there is a space between the cells; this space is known as the segmentation cavity.

The four cells of the animal pole progress in their divisions more rapidly than the four of the vegetable pole, but the latter when the yolk matter is at a minimum, as, for instance, in echinoderms, do not lag much. From their unequal rates of division the two sets of cells come to differ more and more in size, those of the animal pole being much the smaller. The division of the cells take place so that the cells form a continuous layer of epithelium, one cell thick, stretching around the enlarged central segmentation cavity, (Fig. 2) and, the latter being an outside view of an Amphioxus blastula, *cf. infra;* the epithelium consists of a larger area of the small cells of the animal pole, and a small area of the large cells of the vegetable pole. This stage of segmentation is known as the blastula stage; the small cells are destined to form the *ectoderm* of the embryo; the large cells the *entoderm;* the central space is the *segmentation-cavity;* the line along which the two parts of the epithelium (ectoderm and entoderm) join is known as the *ectental line.*

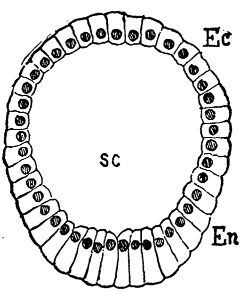


Fig. 2. Blastula of Echinocardium cordatum 20 hours after impregnation *Ec*, ectoderm; *en*, entoderm; *sc*, segmentation cavity. After Selenka.

Vertebrate type of segmentation. In the vertebrates we find that segmentation also results in two epithelia, one ectoderm and one entoderm, joined at their edges, and

surrounding a segmentation-cavity, but the resemblance to the typical blastula is masked by changes in both ectoderm and entoderm; the vertebrate ectoderm when first fully differentiated consists of several layers of cells, and not merely of a single layer of cells as in the primitive type of segmentation; the entoderm contains a very large amount of nutritive material (deutoplasm) and is represented either by a mass of large cells (marsipobranchs, ganoids, amphibians) or a mass of protoplasm, not divided into cells, or but partially divided into cells, and containing an enormous quantity of deutoplasm (sauropsidans and monotremes). In the higher mammals there are further modifications as described below.

The more primitive form among vertebrates is, I think, presumably, that in which the entoderm consists of separate cells, for this mode of segmentation is the one which most resembles that of invertebrates, and it occurs in the lowest vertebrates, and in ova which are not excessively charged with yolk.

In the *primitive form of vertebrate segmentation*, which is preserved in the marsipobranchs, ganoids and amphibians, there is a well marked difference between the cells of the two poles. The following account refers especially to the frog's egg, and is an adaption of Balfour's summary (Comp. Embryl. I., 78, 79). The first formed furrow is vertical; it commences in the upper half of the ovum which corresponds to the animal pole and is characterized by the black pigment—the lower or vegetable pole being whitish. The first furrow extends rapidly through the upper, then more slowly through the lower half of the ovum, so that the divergence in the two polar rates of development is indicated already. As soon as the furrow has cleft the egg into halves, a second vertical furrow appears at right angles to the first and behaves in the same way (Fig. 3). The next furrow is at right angles to both its predecessors, and therefore parallel to the equator of the egg, but it is *much nearer the animal than the vegetative pole.* It extends rapidly around the egg and divides each of the four previous segments into two parts; *one larger with a great deal of yolk, and the other smaller with very little yolk.* The eight segments or cells

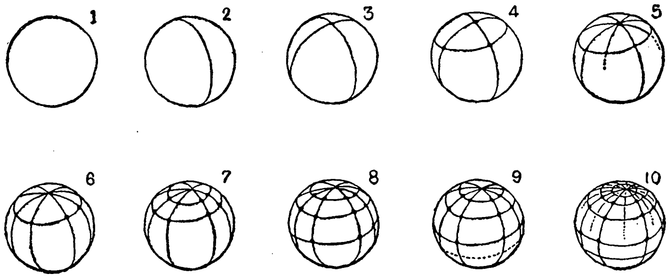


Fig. 3. Segmentation of the egg of the common frog; diagrams slightly modified from Ecker.

have a small segmentation cavity in the centre between them. This cavity increases in size in subsequent stages, its roof being formed by the small cells further divided, and its floor by the large cells, also multiplied by division, though to a less extent than the small cells. All the developmental processes progress more rapidly at the animal pole. After the equatorial furrow, there follows two vertical or meridional furrows which begin at the animal pole and divide each of its four cells into two, making eight small cells. After a short period these furrows extend to the lower pole and divide each of the large cells into two (Fig. 3, 5). The so-called *meridional* cleavages after the first and second are not truly meridional cleavages since they do not pass through the pole of the ovum, but through the poles of the cells, (blastomeres) which they divide; see Rauber, Morph. Jahrb. viii, 287.

A pause now ensues, after which the eight upper cells become divided by a furrow parallel to the equator and *somewhat later* a similar furrow divides the eight lower segments. Each of the small cells is now again divided by a vertical furrow, which later divides also the corresponding large cell. The segmentation cavity is, therefore, now bounded by 32 small and 32 large cells. After this the upper cells (ectoderm) gain more and more in number beyond the lower cells (entoderm). After the 64 segments are formed, two equatorial furrows appear in the upper pole before a fresh furrow arises in the lower, making 128 ectodermal cells against only 32 entodermal.

The regularity of the cleavage cannot be followed further, but the upper pole continues to undergo a more rapid segmentation than the lower. At the close of segmentation the egg forms a sphere, containing an excentric segmentation cavity (Fig. 4, *s. c.*) composed of two unequal parts, an upper arch of several layers of cells, (*Bl.*) the primitive blastoderm of Minot or ectoderm, and a lower mass (*Yolk*) of large cells rich in protoplasm. At the edge of the mass of large cells (*k w*) there is a gradual passage in size to the cells of the blastoderm, and it appears that the small cells receive additions at the expense of the large ones; this zone corresponds to the so-called germinal wall of large vertebrate ova, and also to what we have defined as the ectental line.

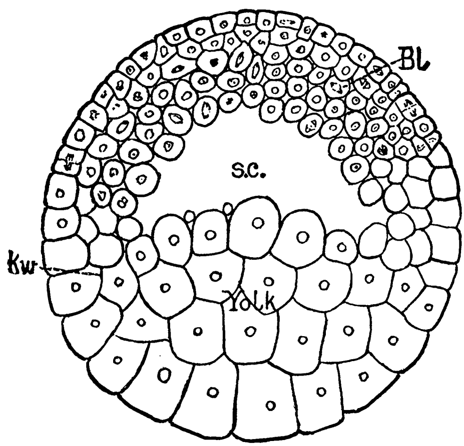


Fig. 4. Section of the segmental ovum axoloth, after Belloni, *Bl.* blastoderm; *s. c.* segmentation cavity; Yolk, yolk or entoderm; *k. w.* (keim wall) germinal wall.

The *secondary type of vertebrate segmentation* differs from the primary principally in the retarded development of the entoderm, due, apparently, to the increase of the yolk-matter. The yolk granules are, as already mentioned, found to be situated not quite exclusively, though almost so, in those parts of the ovum out of which the entodermal cells are formed. Hence, when there is a great deal of yolk the anlage of the entoderm becomes bulky, and when it segments the entodermal cells it produces are correspondingly big, as we have seen is the case in Amphibian ova. On the other hand, when the amount of yolk is small, as in the primitive type of segmentation, *e. g.* echinoderms, the entodermal cells are small. In the reverse case when the amount of yolk is exceedingly great, as in se-

lachians, reptiles and birds, the yolk may not divide into cells as fast as the nuclei multiply, so that it seems that the presence of the deutoplasm, though it does not affect the nuclear divisions markedly, certainly impedes very much the division of the protoplasm, and consequently in these ova we find at certain stages of development a multinucleate yolk. The impediment is not encountered by the protoplasm of the animal pole, hence we see the animal pole segmenting while the yolk does not; in this case the segmentation appears confined to one portion of the ovum, and accordingly such ova are termed *meroblastic* in contradiction to the *holoblastic* ova, in which the first cleavage furrows divide the whole ovum; but the difference, it must be expressly remembered, is one of degree not of kind.

The best known example of a vertebrate meroblastic ovum is, undoubtedly, the hen's egg. The so-called yolk or "yellow" is the ovum; the white and the shell are both adventitious envelopes added by the oviduct as the ovum passes down after leaving the ovary. The segmentation begins while the ovum is passing down through the lower part of the oviduct, and shortly before the formation of the shell commences. If an ovum from the upper part of the oviduct be examined, it is found to be surrounded with more or less white (albumen). Its animal pole is represented by a whitish disk from 2.5—3.5 mm. in diameter and 0.30—0.35 mm. in thickness; this disc is known by many names— formative yolk, germinal disc, cicatricula, (Narbe, Hahnentritt, Keimscheibe, stratum s. discus proligerus). The animal pole consists chiefly of protoplasm and is peculiar only in its small size compared with the whole ovum; it contains, when the ovum leaves the ovary, the egg-cell nucleus; the ovum then matures; impregnation occursand finally segmentation begins. Viewing the ovum from above, we see the first furrow appear as a groove running across the germinal disc, though not for its whole width, and dividing it into halves; this furrow is developed in accompaniment with the division of the segmentation nucleus. The primary furrow is succeeded by a second furrow nearly at right angles to the

first; the surface of the germinal disc is cut up into four segments or quadrants, (Fig. 5, A.) which are not, however, separated from the underlying substance. The number of radiating furrows increases from four, to seven or nine, when there arises a series of irregular cross furrows, by which the central portion of each segment is cut off from the peripheral portion giving rise to the appearance illustrated by Fig. 5, C; there are now a number of small central segments surrounded by large, wedge-shaped external segments. Division of the segments proceeds rapidly by means of furrows running in va-

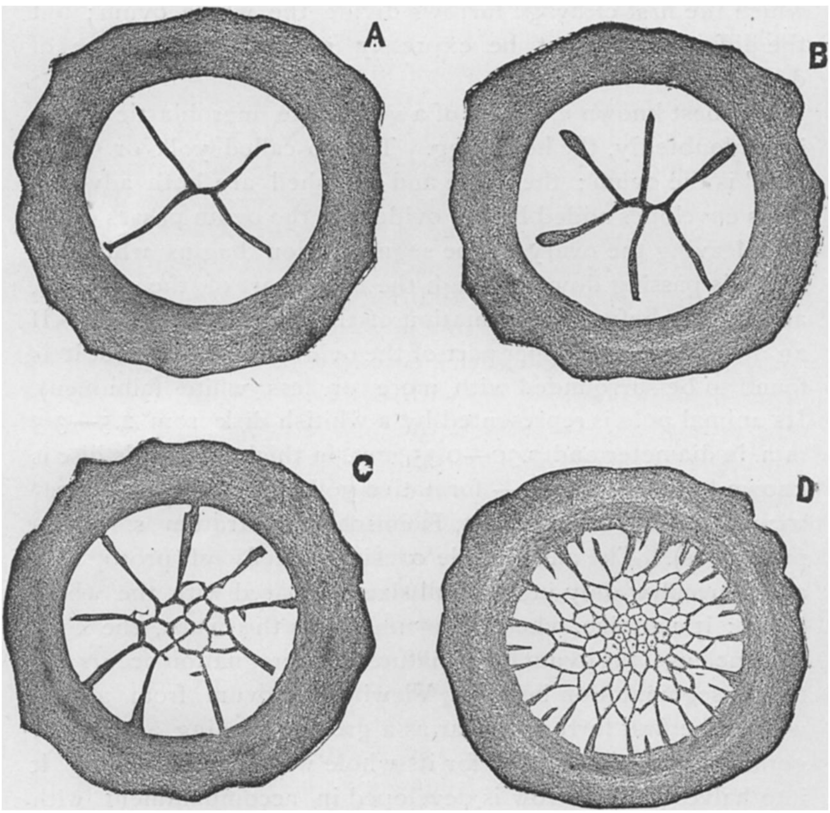


Fig. 5. Four stages of the segmentation of the hen's ovum; after Coste. Only the germinal disc seen from above and part of the surrounding yellow yolk are represented.

rious directions. Not only are the small central segments divided into still smaller ones, (Fig. 5, D.) but their number is increased also by the addition of cells cleft off from the central ends of the large peripheral segments, which are themselves subdivided by additional radiating furrows (Fig. 5, D.). Sections of the hardened germinal disc show that segmentation is not confined to the surface, but extends through the protoplasmic mass of the animal pole, there being deep seated cleavage in planes parallel to the surface of the ovum. According to Duval, **19**, when the first few, small central cells are separated off there is a small space between them and the underlying egg substance (see Figs. 2, 3, 4, 5 and 6 of his Plate I.) and this space he calls the segmentation cavity; but in this, I think, he is in error, for the cells formed below this space are incorporated in the ectoderm or primitive blastoderm; the cells referred to are those marked *im*, in Fig. 8 of Duval's Pl. I. The true segmentation cavity, as we have seen, is bounded on one side by entoderm. This fundamental characteristic Duval has entirely overlooked. From the processes described, there results a disc of cells, which receives peripheral additions; the border from which these additions come is known as the *segmenting zone*. The whole mass of cells derived from the germinal disc represents the ectoderm, and the segmenting zone may be homologized with the cells around the edge of the primitive blastoderm of the frog (Fig. 4, *k w*). A section through the segmented germinal disc shows the following relations: The blastoderm is a disc of cells; its upper layer is epithelioid, its lower layers consists of rounded cells more or less irregularly disposed; at its edge it merges into the yolk which continues to produce cells; between the blastoderm and the yolk is a fissure—the segmentation cavity; the yolk under the fissure contains a few nuclei, which have each a little protoplasm about them, but do not form parts of discrete cells.

In reptiles, the process of segmentation is very similar to that in birds. Our knowledge is based principally upon observations upon the eggs of the European lizards (*Lacerta agilis* and *viridis*) which have been studied by Kupffer and Benecke,

30, Balfour, **2,** Sarasin, **41,** Weldon, **49,** and Hofmann Archives ne'erlandaises xvi, 1881) Hofmann gives a resumé) in Broun's Thierreich vi. Abth. iii. p. 1877-1881. The process is more irregular, and small cells are budded off singly and in scattered clusters from the larger segments. At the close of segmentation the germinal disc is converted into a membrane consisting of several layers of cells and parted from the underlying yolk by a thin space—the segmentation cavity; at its edge this membrane, the primitive blastoderm, is united with the yolk, it being immediately surrounded by a segmentating zone, from which it receives accretions. The layer of the yolk immediately under the segmentation cavity contains scattered nuclei, lying singly or in clusters; each nucleus is surrounded by protoplasm; the nuclei are not all alike; some are very large round with very distinct nuclear threads; others are small and often bizarre in shape; probably the latter are budded off from the former.

In Elasmobranchs, the germinal disc is thicker, and consequently the mass of cells resulting from its segmentation cuts in quite deeply into the yolk, Balfour, Comp. Embryol. i, fig. 46, Rückert, **40,** 28. As segmentation progresses, the cells spread out into a layer, which shows the same essential relations as have been described in birds and reptiles. There is the several-layered primitive blastoderm with its edges connected with the yolk and itself overlying the segmentation cavity, the lower floor of which is formed by the multinucleate yolk the representative of the cellular yolk mass of the frog (Fig. 4, *Yolk*). The nuclei are confined to the layer immediately under the segmentation cavity, and this layer corresponds to the sub-germinal plate in teleost ova. Of the yolk-nuclei some are large, others are small as in reptiles; they are the *Parablast-kerne* of His, the *Merocyten-kerne* of Rückert.

In bony fishes, also, we find the same type, but modified somewhat. The process of segmentation has been very carefully studied by C. O. Whitman, **1,** to whom I am indebted for the accompanying semi-diagrammatic figure of the segmented ovum of a flounder. The ovum is surrounded by a vitelline membrane, *z*, from which it has slightly withdrawn,

notably at the upper pole, where lies the thick cap of cells constituting the blastoderm, *Bl.*; in the stage represented, the outer layer of cells is just beginning to assume an epithelioid character; underneath the blastoderm is the well-marked segmentation cavity, *s. c.*; everywhere at the edge of the blastoderm lies the segmenting zone, *k. w.*, a ring of granular protoplasm with rapidly dividing nuclei; the cells resulting from these divisions are added to the edge of the blastoderm, which thus enlarges peripherally. The protoplasm of the segmenting zone is prolonged inwards forming the floor of the segmentation cavity; this sheet of protoplasm *s. g.*, is known as the *sub-germinal plate*.

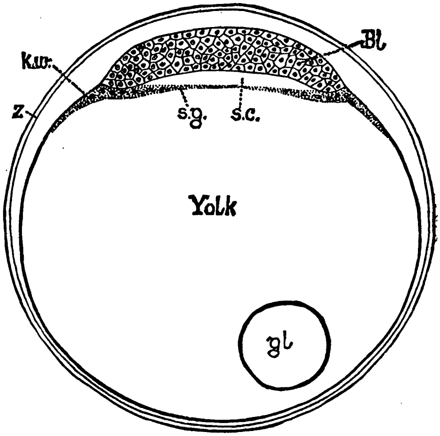


Fig. 6. Ovum of a flounder in transverse vertical section; semi-diagrammatic figure by Dr. C. O. Whitman. *z.* vitelline membrane (or zona); *k.w.* segmenting zone (keim wall); *Bl.* blastoderm or primitive ectoderm; *s. c.* segmentation cavity; *s. g.* sub-germinal plate, *gl.* oil globule of yolk.

The segmenting zone is, of course, the homologue of the similar zone in amniote ova, or the so-called germinal wall, but it is quite sharply defined against the yolk and therein differs from the wall in the chick, because in the latter the germinal merges gradually into the yolk. The process of segmentation differs from that in elasmobranchs and sauropsida, in that the cleavage of the germinal disc is strikingly regular, and further in that the whole width and thickness of the germinal disc is involved in the segmentation from the very start. The segmentation in teleosts is further interesting as affording proof that all the nuclei as shown by Whitman's investigations, arise from the segmentation nucleus.

To summarize: In vertebrate ova with a large yolk which does not divide into cells until segmentation is considerably advanced, the substance of the animal pole segments com-

pletely and produces several layers of cells (the uppermost becoming epithelioid), which are the ectoderm or primitive blastoderm ; the edge of the blastoderm touches the yolk and is surrounded by a nucleated zone in which the production of cells is continuing ; underneath the blastoderm is the fissure-like segmentation cavity ; the floor of this cavity is formed by the unsegmentated yolk (entoderm) which is furnished with scattered nuclei in the layer immediately under the segmentation cavity ; the yolk nuclei, at least in selachians and reptiles, are of two kinds, very large ones and smaller ones, which arise, probably, from the large nuclei ; the nucleated layer may be termed the sub-germinal plate.

Modified segmentation of placental mammals. The lowest mammals resemble the reptiles in many respects ; amongst other reptilian characteristics of the monotremes, we find ova of large size and rich in deutoplasm. That these ova segment during their passage through the oviduct, in similar manner to those of reptiles, was first ascertained by direct observation by Caldwell in 1884, **16.**

In marsupials and the placental mammalia the amount of yolk substance is greatly reduced and the ovum is of small size. It is, therefore, holoblastic, that is to say, the cleavage planes cut through the entire cell, as in the primitive type of segmentation, but the arrangement of the cells at the close of segmentation appears to be a direct inheritance from the reptilian ancestors of the mammals.

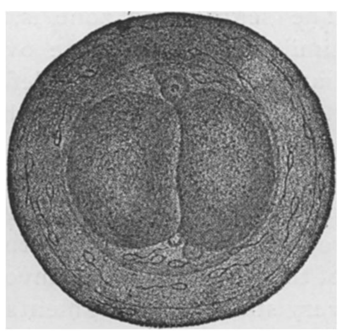
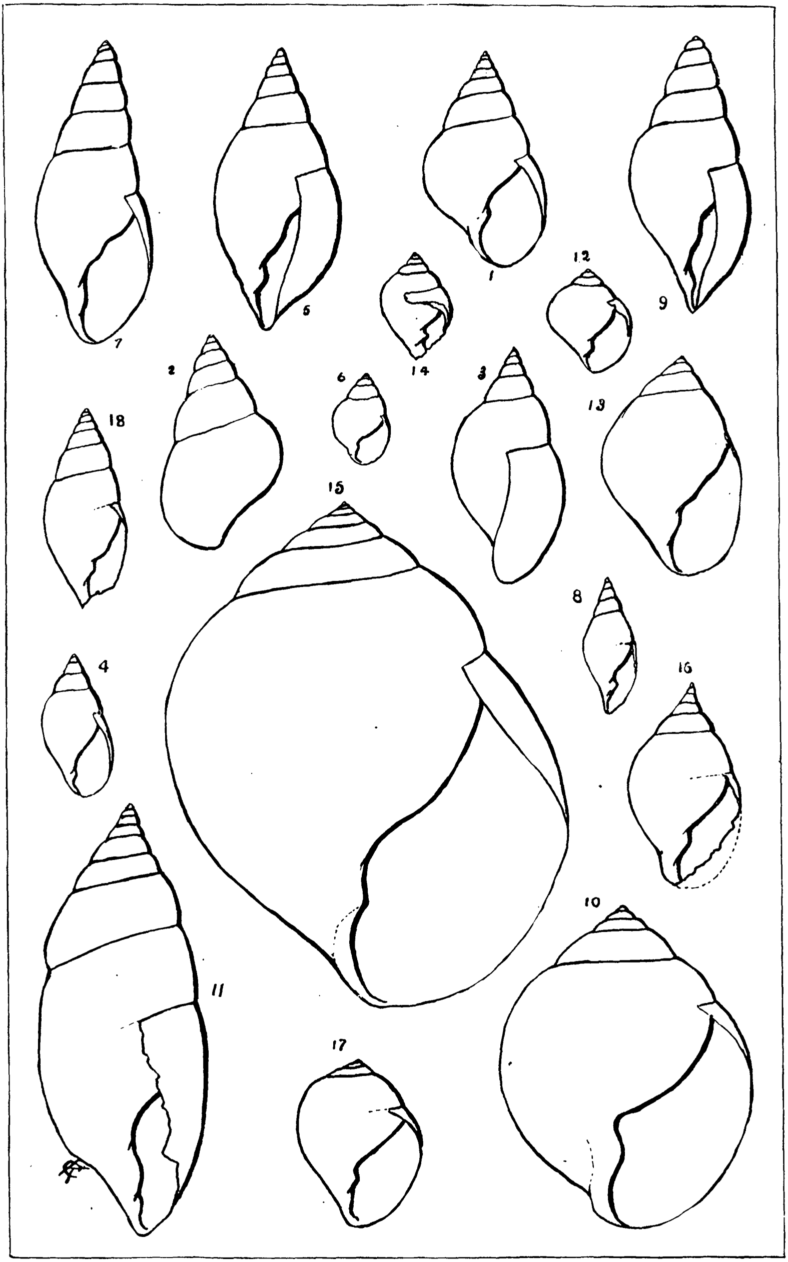


Fig 7. Ovum of a rabbit of twenty-four hours; after Coste. The first cleavage has been completed; the two cells are appressed; above the cells lie the polar globules; numerous spermatoza lie in and within the zona pellucida.

The segmentation of the mammalian ovum was first clearly recognized by Bischoff, though it had been previously seen and misinterpreted by Barry, **5, 6, 7**; very beautiful figures of segmentation in the rabbit have been given by Coste, **17.** More recently, observations have been published by Hensen on the rabbit, **24,** van Beneden on the rab-

PLATE XX.



Soleniscus.

bit, **8, 9, 10,** Kupffer on rodents, **29,** Selenka on rodents, **44, 45, 46,** and oppossums, **47,** van Beneden and Julin on bats, **13,** Heape on moles, **23,** Tafani on white mice, **48.**

The ovum, when discharged from the ovary, is surrounded by the corona radiata, which is lost when impregnation takes place. Segmentation begins when the ovum is one-half to two-thirds of the way through the oviduct. The ovum spends about 70 hours in the oviduct in the rabbit, and about eight days in the dog. The first cleavage plane passes through the axis of the ovum which is marked by the polar globules. When first formed, the two segmentation spheres are oval and entirely separated from one another, but subsequently they flatten against one another and become appressed—a remarkable phenomenon of which we possess no explanation whatever. The second cleavage plane is also meridional.

The ovum next divides into eight and then into twelve segments, of which four are larger than the rest.

The succeeding cleavages have never been followed accurately, but from Heape's observations on the mole, **23,** 166, we know that the divisions progress with great irregularity, and is probable that the commonly assumed regularity of mammalian segmentation does not exist in nature. After a time (in the rabbit about 70 hours) there is reached the stage termed *Metagastrula* by van Beneden, **10,** 153-160, in accordance with his view of the homologies of this stage. The metagastrula consists of a single layer of cuboidal hyaline cells lying close against the zona pellucida (Fig. 8) *en;* the space within this layer contains an inner

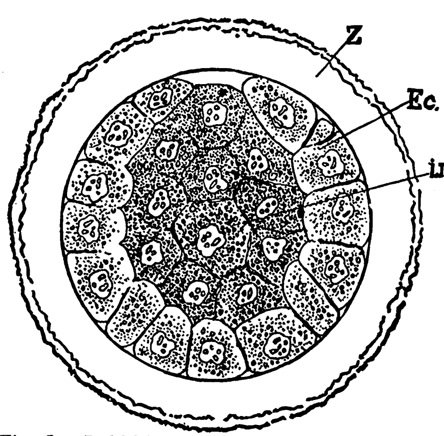


Fig. 8. Rabbit's ovum of about 70 hours; after E. van Beneden. *z.* Zona pellucida; *en.* [entoderm; *i. m.* inner mass of granular cells.

mass of cells, *im*, which are rounded or polyzonal and densely granular. At one point the outer layer is interrupted and the space is filled by *one* of the granular segments of the inner mass (Fig. 8). The nuclei of all the cells are somewhat nodulated, and have several highly refractible granules each. The granules in the bodies of the cells of the outer layer are somewhat concentrated around the nucleus, leaving the cortices of the cells clear, van Beneden, **9**, 28-29, has observed that sometimes (21 oval out of 29) the first two segmentation spheres are of unequal size in the rabbit, and similar variability occurs in the mloe, Heape, **23**, 165 ; Tafani, on the other hand, expressly denies its occurence in white mice. It is, I think, very improbable that this difference, which sometimes occurs and sometimes does not, has any fundamental significance ; van Beneden, however, has maintained that the small cell gives rise in the rabbit to the inner mass of cells, (see below) which he terms the entoderm, but which must, it seem to me, be homologized with the ectoderm, as explained below. That van Beneden is in error, as to the genetic relation of the small cell to the inner mass has been demonstrated by Heape, **23**, 166.

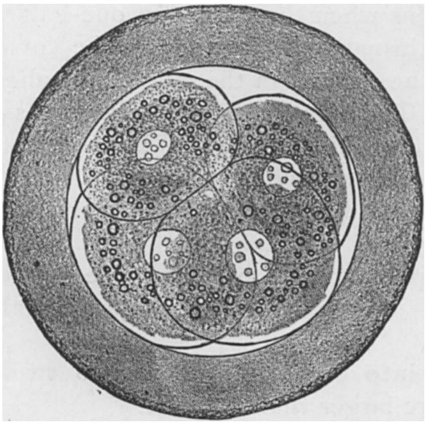


Fig. 9. Ovum of a bat, Vespertilio murina, with four segmentation spheres ; after van Beneden and Julin.

The second cleavage plane is probably also meridional, and is certainly at right angles to the first, so that four similar cells are produced as in the primitive type of segmentation,[1] (Fig. 9) those four cells are also rounded at first, and probably become fitted against one another so as to produce the

[1] The distinction here made between "primitive type of segmentation" an "primitive type of *vertebrate* segmentation" should be borne in mind by the reader.

disposition observed by Tafani 1889, **48**, 116, in mice ova at this stage; Tafani describes each cell as having the form of a three-sided pyramid with the apex at the centre of the ovum and a convex base forming part of the external surface of the yolk. That the two first cleavage planes are meridional is rendered probable by the arrangement in the four cell stage observed by Selenka in the Virginian oppossum. (Fig. 10.)

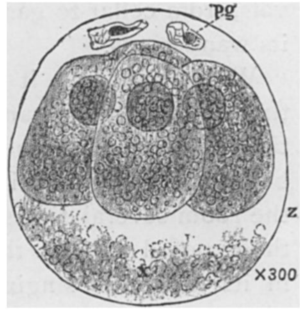


Fig. 10. Ovum of Virginian oppossum with four segments; after Selenka.

(To be continued.)

THE SONG OF THE SINGING MOUSE.

BY. WM. T. DAVIS.

IN the daily papers and in scientific journals references to singing mice are not uncommon, some relating to wild species, but generally to the house mouse. The authors of these notices usually refer to the mice as singing from happy choice, as if they greatly enjoyed their own music, and in captivity, they have been reported as singing when food was given them, or when turning the wheel, as expressive of delight and high spirits. In some cases a mouse may be able to sing at will, but I think, from the descriptions I have read, that it is generally involuntary, as it certainly was in the individual that came under my own observation.

Several years ago, in November, I heard a strange noise near some water pipes in a store room, and at first thought that one of them had broken, and that a little stream was gurgling between the walls. However, later on, this gurgling noise was found to be produced by a mouse, which ran from behind various boxes as they were, in turn, removed, keeping up a constant song. A trap was set, and after a few days the mouse was captured. In the meantime, it was heard at inter-